

Rapid Sample Preparation and Identification of Infectious Microorganisms using Matrix-assisted Laser Desorption/Ionization and Time-of-flight Mass Spectrometry

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Introduction:

The rapid detection of unknown infectious microorganisms in biological and environmental samples is a public health priority. The current Polymerase Chain Reaction (PCR) detection methods used in Public Health Laboratories are time consuming and results are not available for 24-48 hours after sample preparation. Matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF/MS) has been shown to be a much more powerful technology for the rapid screening for infectious microorganisms in biological samples. Previous work in this area has shown that it is possible to make distinctions at the strain level of microorganisms using a variety of MALDI-TOF/MS approaches. Studies have been performed to identify *Escherichia coli* strains based on ribosomal subunits obtained from whole cell lysates [1]. Whole cell approaches to differentiating between strains of microorganisms have been utilized with *E. coli* spp [2,3], *Haemophilus* spp[4], and *Staphylococcus* spp[5]. Screening for unknown infectious microorganisms is aided by the development of libraries of known microorganism spectra as well as pattern recognition software [6] and algorithm improvements [7] in recent years. The use of MALDI-TOF/MS is ideally suited for rapid screening of unknown infectious microorganisms due to the minimal amount of time needed for sample preparation prior to analysis.

Experimental:

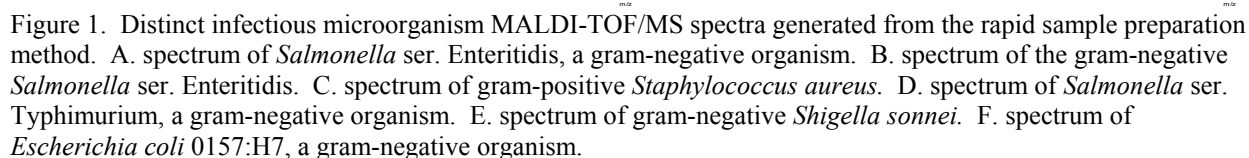
Sample preparation was limited to the minimum necessary for inactivation of the infectious organism and brief clean up to remove major interferences⁵. After isolation bacteria were streaked onto TSA 5% sheep blood plates and incubated at 37°C for 16 hours. The bacterial sample was washed three times with 500 µl sterile water. The pelleted bacteria were suspended in 150 µl 50% Acetonitrile (ACN) + 0.1% Trifluoroacetic acid (TFA). Gram-positive bacteria were enzymatically digested 1:1 (v/v) with lysozyme (1mg/ml) for 30 minutes in a 37°C sonicating water bath. An aliquot of digested gram-positive bacteria or undigested gram-negative bacteria was mixed 1:1 (v/v) with Sinapinic acid (SA) matrix and spotted onto a target. The optimal number of peaks were produced with a Bruker Daltonics Ultraflex instrument (Bruker Billerica, MA) using the following parameters: linear acquisition mode, PIE delay of 350 nsec, positive ion mode, ion source voltage 1 25 kV, ion source voltage 2 23.40 kV, ion source lens voltage 6.00 kV, laser repetition rate in Hz 50 psec, linear detector voltage 1.65 kV, and 100 laser shots. The resulting spectra were processed using flexAnalysis.

Results:

The spectra generated strongly suggest that speciation of microorganisms is possible (Figure 1). The spectra obtained from the standard strains indicated that significant differences are observed for various strains and substrains, i.e. *Salmonella* ser. Enteritidis and *Salmonella* ser. Typhimurium (Figure 1A, B and D respectively); it is these distinctions that allowed for speciation. *Salmonella* ser. Enteritidis has unique peaks at 6146.8, 7726.3, 8397.2, 9602.3, 11049.3, and 11258.0 amu. *E. coli* has unique identifying peaks at 4374.6, 6272.8, 6333.0, 6428.8, 9572.9, and 16148.5 amu. *Staphylococcus aureus* unique peaks are 1070.8, 1294.4, 1517.6, 1799.2, 2022.5, 4775.1, 6902.5, and 9659.7 amu. *Salmonella* ser. Typhimurium unique peaks are 2527.5, 3226.0, 5002.4, 5409.6, 6140.3, 6367.0, 7726.7, 8400.9, 11052.0, and 12291.4 amu. *Shigella sonnei* has unique peaks at 4167.5, 4535.2, 4873.9, 6863.6, 7278.2, 7873.2, 8332.3, 9071.1, 9542.6, 9745.7, 9952.3, 10482.2, 11228.9, 11986.0, and 15413.2 amu. Significant spectral differences were observed between the gram-negative and gram-positive microorganisms as well (Figure 1A, B, D, E, and F [-] versus C [+] respectively). A library of mass spectral fingerprints is being compiled for species of *Escherichia*, *Staphylococcus*, *Shigella*, and *Salmonella* in preparation for identification of unknown infectious microorganism spectra.

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